

SHORT COMMUNICATION

***Beauveria bassiana* infection of eggs of stored-product beetles**

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Abstract

Beauveria bassiana (Balsamo) Vuillemin was tested under maximum challenge conditions with an estimated dose of 1.1×10^5 conidia/mm² for its effects on eggs of four of the major beetle pests of stored grain and grain products. When ambient relative humidity (RH) was 92%, hatch of fungus-treated *Rhyzopertha dominica* (Fabricius) eggs was 13% versus 58% for control eggs, and hatch of treated *Tribolium castaneum* (Herbst) was 17% versus 51% for controls. There was no fungus effect at RH of 48 and 73%. Fungus treatment of *Cryptolestes ferrugineus* (Stephens) and *Oryzaephilus surinamensis* (Linnaeus) eggs had no effect. Sectioned *T. castaneum* eggs demonstrated that the fungus penetrates and infects them.

Key words: *Beauveria bassiana*, Coleoptera, egg infection, stored products.

Introduction

Beauveria bassiana (Balsamo) Vuillemin has a broad host range for arthropods, but reports of its infectivity for eggs vary from none to highly infective. Several reports confirm the susceptibility of tick, Hemiptera and Lepidoptera eggs (Lynch & Lewis 1978; Kaaya & Hassan 2000; Ekesi *et al.* 2002). Attempts to infect Coleoptera eggs have produced mixed results. Marannino *et al.* (2006) reported a significant reduction in egg hatch for the buprestid *Capnodis tenebrionis* (Linnaeus) treated with *B. bassiana*. Silva *et al.* (2006) reported higher mortality for *B. bassiana*-treated eggs of the tenebrionid *Alphitobius diaperinus* (Panzer) than for other stages of the insect, and Chikwenhere and Vestergaard (2001) infected eggs of the weevil *Neochetina bruchi* Hustache. On the other hand, Poprawski *et al.* (1985) reported that *B. bassiana* was not effective for infecting eggs of the weevils *Otiorrhynchus sulcatus* Fabricius and *Sitona lineatus* Linnaeus. Similarly, Hedlund and Pass (1968) found that eggs of the alfalfa weevil *Hypera postica* (Gyllenhal) were not susceptible, and Long *et al.* (1998) reported that *B. bassiana* did not reduce the hatch rate nor the first instar viability of *Leptinotarsa decemlineata* (Say).

There are no reports of fungus effects on the eggs of stored-product pests. This study was intended to determine whether the eggs of major beetle pests of stored products could be infected with a maximum challenge from *B. bassiana*.

Materials and methods

Beetle eggs were obtained from overnight oviposition of colonies of eastern Kansas origin that have been maintained at the USDA Grain Marketing and Production Research Center, Manhattan, KS, USA for several years without wild input. *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (Fabricius), *Oryzaephilus surinamensis* (Linnaeus) and *Cryptolestes ferrugineus* (Stephens) eggs from 24 h oviposition were placed individually in wells of Polysorp 96-well plates (Nunc, Naperville, IL, USA). Eggs in 24 wells on one side of each plate were dusted and eggs in 24 wells on the opposite side were a control replicate. Three replicates of each test were done over time to avoid pseudoreplication. The eggs were dusted with commercially produced conidia of *B. bassiana* isolate GHA (Laverlam, Butte, MT, USA). The conidia were placed in a 7.5 mL vial that was covered

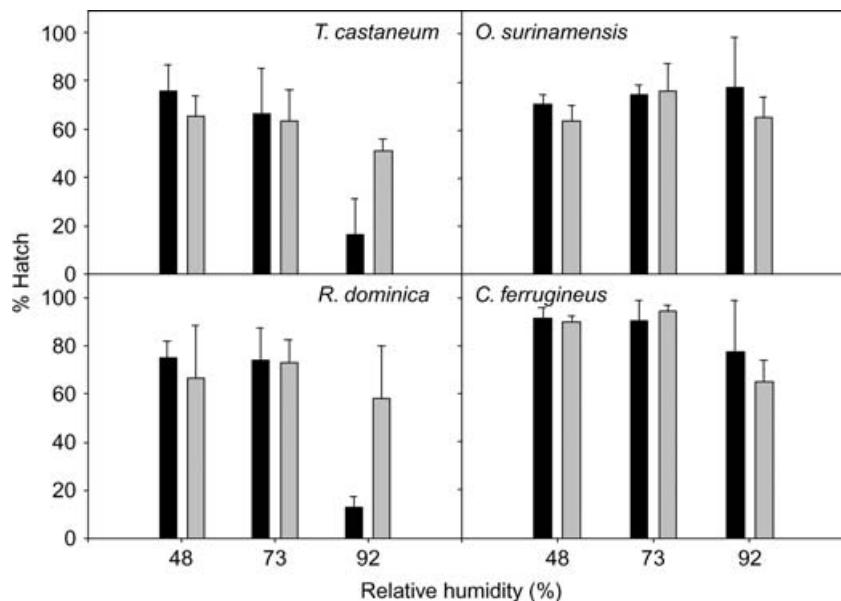


Figure 1 Percentage hatch of beetle eggs after exposure to *Beauveria bassiana* (■) at three relative humidities. □, Control.

with nylon mesh with 0.2 mm openings through which they were dispensed by gentle tapping. To obtain a dose estimation, glass slide cover slips were dusted by the same method that was used for the eggs. The conidia were then washed from the coverslips by vortexing in 0.1% Silwet L-77 (Loveland, Greeley, CO, USA) and counted with a hemacytometer. The dose estimate was 1.1×10^5 conidia/mm² ($SD = 0.25 \times 10^5$). The conidia had a 93% germination rate after 18 h at 26°C.

The plates were incubated at 26°C in plastic bins with saturated K₂CO₃, NaCl or water to achieve 48, 73 or 92 ± 1% relative humidity (RH), respectively. Incubations were continued through cessation of egg hatch, and the eggs were checked at 2 day intervals for hatch.

SigmaStat 3.1 (Systat, Point Richmond, CA, USA) was used for *t*-tests and ANOVA.

For histology, *T. castaneum* eggs were fixed in 2.5% formaldehyde 5 days after exposure to *B. bassiana* conidia, dehydrated with an alcohol series, embedded in Spurr's resin, sectioned to 2 µm, and stained with paragon stain.

Results and discussion

Significantly reduced egg hatch with *B. bassiana* exposure occurred only at 92% RH, the highest tested, and only with *R. dominica* ($t = 3.5$, d.f. = 4, $P = 0.025$) and *T. castaneum* ($t = 3.9$, d.f. = 4, $P = 0.018$) (Fig. 1). Eggs of *R. dominica* but no other species occasionally became reddened after *B. bassiana* exposure. This is presumably due to the pigment oosporein and confirms infection. Infection of *T. castaneum* eggs was also confirmed by the presence of mycelium in sections (Fig. 2). There was no significant effect on either

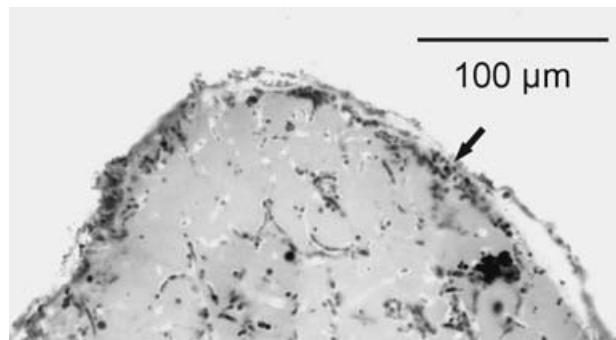


Figure 2 Section of *Beauveria bassiana*-infected *Tribolium castaneum* egg showing mycelium as black dots and lines throughout the tissue and as a mass under the chorion (arrow).

C. ferrugineus or *O. surinamensis* at any of the three humidities. Higher mortality in *R. dominica* eggs than in those of the other species may be a result of the 8 day pre-hatch interval. Eggs of all of the other species began to hatch within 6 days of oviposition at 26°C. The longer interval would provide an extended period for the fungus to penetrate the egg and infect the embryo. Although suboptimal moisture for egg development may have contributed to the differential effect of *B. bassiana* on these species, the data are not conclusive.

There was a greater than 10% reduction of hatch in control eggs with *R. dominica* and *T. castaneum* at 92% RH as compared with either 72 or 42% RH, but the differences were not significant ($F = 0.5$, d.f. = 2.6, $P = 0.63$ and $F = 2.02$, d.f. = 2.6, $P = 0.21$, respectively). Hatch reduction with 92% RH for *C. ferrugineus* or *O. surinamensis* did not exceed 7%.

It is not clear why the eggs of some insects are susceptible to fungus while others tolerate maximum challenge. It has been demonstrated that *Manduca sexta* (Linnaeus) embryos have the ability to respond to microbes with immune responses (Gorman *et al.* 2004). Such responses may be partially responsible for the difficulty of infecting eggs, but the barrier presented by the egg chorion is a formidable first defense and is probably the major factor. Once hatched, the neonate larvae are highly susceptible to fungus. When exposed to the fungus, newly hatched larvae of all four beetle species were rapidly killed.

This study was carried out in a manner that favored the development of fungal infection in order to determine not only whether there was infectivity of eggs of the target insects under operational conditions but also to determine whether tolerant eggs are impervious to infection under maximum assault. The temperature was near the optimum for *B. bassiana* growth (Fargues *et al.* 1997), but not for the beetles, which develop fastest at temperatures of 30 to 35°C (Howe 1965). While relative humidities of 48 and 73% are common in the habitats of stored-product insects, 92% is greater than is normally encountered, and it may be stressful to the insects. In some cases, there was external fungal growth on eggs, especially when grain or flour residue was present. While *B. bassiana* can attack *R. dominica* and *T. castaneum* with a maximum challenge dose and high relative humidity, the reduced hatches that are reported for *R. dominica* and *T. castaneum* would be rare in nature and cannot be considered a factor in mycological pest control. It must be concluded that the direct effect on eggs would have little or no impact on *B. bassiana* efficacy as a control agent for the four beetle species in this study.

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Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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